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(57) Abstract

The invention relates to a stabilised immunoglobulin composition comprising at least one immunoglobulin together with a stabilising amount of a chelator of copper ions such as EDTA or citrate. Preferably the immunoglobulin is an antibody, for example a recombinant CDR-grafted antibody against the CDw52 antigen, most preferably CAMPATH-1H. The invention also relates to a process for enhancing the stability of an immunoglobulin which comprises subjecting the immunoglobulin to a purification procedure capable of removing copper ions therefrom. Preferably the immunoglobulin is rendered substantially free from detectable copper ions, for example on atomic absorption spectroscopy.

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STABILISED ANTIBODIES

The present invention relates to the stabilisation of immunoglobulins against degradation, in particular on storage and processing prior to use.

Antibodies or immunoglobulins are proteinaceous bifunctional molecules. One part, which is highly variable between different antibodies, is responsible for binding to an antigen, for example the many different infectious agents that the body may encounter, whilst the second, constant, part is responsible for binding to the Fc receptors of cells and also activates complement. In this way, antibodies represent a vital component of the immune response of mammals in destroying foreign microorganisms and viruses.

The immunisation of an animal with an antigen results in the production of different antibodies with different specificities and affinities. An antiserum obtained from the immunised animal will, therefore, be heterogeneous and contain a pool of antibodies produced by many different lymphocyte clones. Antibodies thus obtained are referred to as polyclonal antibodies and this polyclonal nature has been a major drawback in the use of antibodies in diagnostic assays and in therapeutic applications.

A major step forward occurred in 1975 when Kohler and Milstein (Nature, 1975, 256, 495-497) reported the successful fusion of spleen cells from mice immunized with an antigen with cells of a murine myeloma line. The resulting hybrid cells, termed hybridomas, have the properties of antibody production derived from spleen cells and of continuous growth derived from the myeloma cells. Each hybridoma synthesizes and secretes a single antibody to a particular determinant of the original antigen. To ensure that all cells in a culture are identical, i.e. that they contain the genetic information required for the

synthesis of a unique antibody species, the hybridomas resulting from cell fusion are cloned and subcloned. In this way, the cloned hybridomas produce homogeneous or monoclonal antibodies.

The advantages of hybridoma technology are profound. Because many hybrids arising from each spleen are screened for their potential to produce antibodies to the antigen of interest and only a few are selected, it is possible to immunize with impure antigens and yet obtain specific antibodies. The immortality of the cell line assures that an unlimited supply of a homogeneous, well-characterised antibody is available for use in a variety of applications including in particular diagnosis and immunotherapy of pathological disorders. Unfortunately, the usefulness of such monoclonal antibodies in a clinical setting can be severely hampered by the development of human anti-mouse antibodies - an anti-globulin response - which may interfere with therapy or cause allergic or immune complex hypersensitivity. This has led to the development of humanised antibodies.

An antibody molecule is composed of two light chains and two heavy chains that are held together by interchain disulphide bonds. Each light chain is linked to a heavy chain by disulphide bonds and the two heavy chains are linked to each other by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains, and each light chain has a variable domain at one end and a constant domain at the other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The remaining constant domains of the heavy chains are aligned with each other. The constant domains in the light and heavy chains are not involved directly in binding the antibody to the antigen.

The variable domains of each pair of light and heavy

chains form the antigen binding site. They have the same general structure with each domain comprising a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases comprising part of, the beta-sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site.

In the use of murine monoclonal antibodies, the induction of an human anti-mouse antibody response is due to the murine origin of the constant domains and four framework regions. This problem has therefore been addressed by the development of modified antibodies of two basic types. The first type, referred to as chimeric antibodies, is where the murine constant domains only are replaced by equivalent domains of human origin (Morrison et al, P.N.A.S., 1984, 81, 6851-6855; Boulianne et al, Nature, 1985, 314, 268-270; and Neuberger et al, Nature, 1985, 314, 268-270). The second type is where the murine constant domains and the murine framework regions are all replaced by equivalent domains and regions of human origin. This second type of modified antibody is referred to as a humanised or CDR-grafted antibody (Jones et al, Nature, 1986, 321, 522-525; and Riechmann et al, Nature, 1988, 332, 323-327).

To generate sufficient quantities of antibody for full clinical investigation, it is desirable to utilize an efficient recombinant expression system. Since myeloma cells represent a natural host specialized for antibody production and secretion, cell lines derived from these have been used for the expression of recombinant antibodies. Often, complex vector design, based around immunoglobulin gene regulatory elements, is required, and final expression levels have been reported which are highly

variable (Winter et al, Nature, 1988, 332, 323-327; Weidle et al, Gene, 1987, 60, 205-216; Nakatani et al, Bio/Technology, 1989, 7, 805-810; and Gillies et al, Bio/Technology, 1989, 7, 799-804).

Other types of expression systems which have been proposed for antibodies include immortalised human B cells (Rice et al, Proc. Natl. Acad. Sci. USA, (1982) 79 7862-7865), however yields are generally low and it is difficult to establish stable cell lines. E. coli has been used to express F_v fragments (Skerra & Plukthun, Science, (1988) 240, 1038-1041) or single chain antigen binding molecules (Bird et al, Science, (1988) 242, 423-426) but entire immunoglobulins have so far not been produced in the system. Antibodies have, however, been successfully produced in mammalian cell expression systems which are already known for the production of recombinant proteins such as Chinese hamster ovary (CHO) cells.

In the production of purified antibodies whether for therapeutic or diagnostic use, it is important that the antibody is sufficiently stable on storage and various chemical entities may have an adverse effect on the stability of the antibody. The present invention is based on the surprising discovery that trace amounts of copper (Cu^{++}) have a destabilising effect on immunoglobulin molecules on storage and that this effect can be eliminated by formulating the immunoglobulin molecule with a suitable chelator of copper ions.

It has also surprisingly been found that the presence of a chelator of copper ions may have a stabilising effect on the immunoglobulin molecule even when the immunoglobulin does not contain amounts of copper which are detectable by conventional techniques such as atomic absorption spectroscopy. Whilst not wishing to be bound by any particular theory, it may be that the presence of copper ions in amounts below the detection limits of techniques such as atomic absorption spectroscopy still has a

destabilising effect on the immunoglobulin molecule which can be eliminated by the addition of a suitable chelating agent.

The present invention provides a stabilised immunoglobulin composition comprising at least one immunoglobulin together with a stabilising amount of a chelator of copper ions.

The invention also provides the use of a chelator of copper ions to stabilise an immunoglobulin against degradation on storage, for example degradation resulting from the effect of copper ions.

The fact that trace amounts of copper ions have a destabilising effect on immunoglobulins means that there may be an advantage in terms of stability in ensuring that immunoglobulins contain the minimum possible amount of copper ions. According to a further aspect the present invention provides a purified immunoglobulin substantially free from copper ions. In particular the invention provides an immunoglobulin in which no copper can be detected by the use of conventional techniques such as atomic absorption spectroscopy.

The invention also provides a process for enhancing the stability of an immunoglobulin which comprises subjecting the immunoglobulin to a purification procedure capable of removing copper ions therefrom. In particular the procedure should be such that the no copper can be detected in the immunoglobulin by the use of conventional procedures such as atomic absorption spectroscopy. Copper can be removed from immunoglobulins by conventional procedures known in the field of protein purification such as dialysis versus potassium cyanide containing phosphate buffer followed by gel filtration to remove copper as copper cyanide (see for example Baker and Hultquist, J. Biol. Chem., 253, 844-845 (1978)).

The present invention is applicable to the stabilisation of immunoglobulins of all classes, i.e IgM,

IgG, IgA, IgE and IgD, and it also extends to the stabilisation of Fab fragments and bispecific antibodies. The invention is preferably applied to the stabilisation of immunoglobulins of the class IgG, which includes the sub-classes IgG_1, IgG_{2A}, IgG_{2B}, IgG_3 and IgG_4. The invention is more preferably applied to the stabilisation of immunoglobulins of the class IgG_1.

The invention finds particular application in the stabilisation of recombinant antibodies, most particularly chimeric antibodies or humanised (CDR-grafted) antibodies. Particular examples of these include chimeric or humanised antibodies against CD2, CD3, CD4, CD5, CD7, CD8, CD11a,b, CD18, CD19, CD25, CD33, CD54 and especially humanised antibodies against the CDw52 antigen, such as CAMPATH-1H (CAMPATH is a Trade Mark of the Wellcome group of companies). Further examples include chimeric or humanised antibodies against various tumour cell marker antigens.

The immunoglobulin will generally be formulated with the metal ion chelating agent at an early stage, for example during or immediately following purification. The production procedure for an immunoglobulin will generally involve purification by means of chromatography and/or gel filtration columns. The chelating agent can be added at any convenient stage of the purification procedure, for example at the stage of the final column, so that the chelating agent remains in the immunoglobulin at the end of the purification procedure. Alternatively, the chelating agent may be added at a suitable stage following purification. In the case of a lyophilised immunoglobulin the chelating agent will generally be added prior to lyophilisation.

The level at which the chelating agent is added to the immunoglobulin will be such as to ensure that any copper present is bound by the chelating agent and thus rendered ineffective in destabilising the immunoglobulin. The invention is applicable irrespective of the intended

end use of the immunoglobulin although the chelating agent which is used should be chosen in such a way that it will not have an adverse effect on the intended end use of the immunoglobulin. For example in the case of antibodies intended for therapeutic use, the chelating agent should not show any toxic effects at the level in which it will be present.

A particularly preferred metal ion chelating agent is ethylenediamine tetraacetic acid (EDTA) which may typically be added to the immunoglobulin at levels of 0.05mM to 5mM, preferably 0.1mM to 3mM. A level of 0.1mM EDTA will often be sufficient to stabilise an immunoglobulin but levels up to 2mM or higher do not present any problem physiologically in the case of an immunoglobulin intended for administration to humans. An alternative metal ion chelating agent is citrate ion, preferably used in the form of an alkali metal citrate, e.g. sodium citrate.

Immunoglobulins intended for therapeutic use will generally be administered to the patient in the form of a pharmaceutical formulation. Such formulations preferably include, in addition to the immunoglobulin, a physiologically acceptable carrier or diluent, possibly in admixture with one or more other agents such as other immunoglobulins or drugs, such as an antibiotic. Suitable carriers include, but are not limited to, physiologic saline, phosphate buffered saline, phosphate buffered saline glucose and buffered saline. Alternatively the immunoglobulin may be lyophilised (freeze dried) and reconstituted for use when needed by the addition of an aqueous buffered solution as described above. Routes of administration are routinely parenteral, including intravenous, intramuscular, subcutaneous and intraperitoneal injection or delivery. The chelating agent may be incorporated into any type of immunoglobulin formulation intended either for storage and distribution or ultimate use. The pharmaceutical formulation will

generally contain, or in the case of a lyophilised preparation will be reconstituted to contain, an effective therapeutic dose of the immunoglobulin per unit dose. In the case of the humanised antibody CAMPATH-1H, liquid formulations or reconstituted lyophilised formulations preferably contain 0.5 to 20 mg/ml of the antibody, preferably 2 mg/ml or 10 mg/ml.

The invention is illustrated by the following examples:

EXAMPLE 1

The effect of various additives on the stability of a recombinant antibody was studied at 37°C. The antibody was CAMPATH 1H, a humanised antibody against the CDw52 antigen (Riechmann et al, Nature, 322, 323-327 (1988)), which had been produced by expression in a recombinant CHO cell line transformed with DNA encoding the heavy and light chains of the antibody molecule. The antibody was extracted from the cell culture medium and purified and was then stored as a solution (1mg/ml) in phosphate buffered saline at +4°C.

Vials containing 0.5ml of the solution of CAMPATH 1H referred to above together with the additive specified were incubated at +37°C for 4 weeks under sterile conditions. At the end of this period the samples were analysed by size exclusion HPLC, the stability of the sample being assessed by the extent of the formation of "peak C" (a peak formed by the major degradation product of the antibody which has a molecular weight of about 50K) based on the total eluted protein. The results are set out in the following Table 1.

TABLE 1

ADDITIVE	% PEAK C
None	12%
None (storage at +4°C)	2%
Cu^{++} (10ppm)	28%
EDTA (2mM)	<1%
1,10-phenanthroline (10mM)	3%

The copper was added as $CuCl_2.2H_2O$ and the 1,10-phenanthroline as a solution in water containing 2% (v/v) ethanol.

These results demonstrate that copper enhances the degree of degradation of the antibody relative to the control. The addition of EDTA virtually eliminates degradation whilst the other metal ion chelator 1,10-phenanthroline reduces degradation to a considerable extent.

EXAMPLE 2

This example also used CAMPATH 1H produced in CHO cells of the type referred to in Example 1 (11.3mg/ml in phosphate buffered saline) and the batch having been measured as containing 0.04μg Cu^{2+}/ml. In this and following examples, the copper content of antibody samples was measured by atomic absorption spectroscopy using a Philips PU9400X atomic absorption spectrophotometer. The detection limit of this method was about 0.03 μg Cu/ml so that samples stated to have "no detectable copper" contain less than 0.03 μg Cu/ml. Samples of this Campath 1H were diluted to 1 mg/ml in phosphate buffered saline and dialysed exhaustively versus 0.2M sodium phosphate buffer at pH 6.0, pH 6.4 and pH 6.8, CAMPATH 1H previously having been determined to be most stable against degradation by

heat at about pH 6. The following was added to 300µl samples at each pH:

(i) 30µl 10mM $CuCl_2.2H_2O$ in water;

(ii) 30µl 10mM EDTA in water;

(iii) 30µl buffer;

and the samples incubated at 62°C for 24 hours. 50 µl aliquots were analysed as described in Example 1 with degradation being assessed by size exclusion chromatography and measured as the extent of formation of "Peak C" based on the total eluted protein.

The results for % Peak C are given in Table 2 below:

TABLE 2

pH	% Peak C		
	Cu	EDTA	Buffer
6.0	1.75	0.38	0.69
6.4	2.94	0.34	0.72
6.8	5.31	0.51	1.12

The results indicate that as pH increases, the effect of copper on the degradation of CAMPATH 1H increases. In the absence of added copper an increase in % Peak C is also seen with increasing pH. In the presence of EDTA the degradation of CAMPATH 1H is suppressed.

EXAMPLE 3

This example used two different batches of CAMPATH 1H produced in CHO cells of the type referred to in Example 1 (10mg/ml in phosphate buffered saline): Batch 1 contained no detectable Cu^{2+} as determined by atomic absorption spectroscopy and Batch 2 contained 0.04µg Cu^{2+}/ml. Samples of both batches were diluted to 1 mg/ml in phosphate buffered saline and dialysed extensively for 24 hours at +4°C against 50mM ammonium hydrogen carbonate and 1mM EDTA

was added to the Batch 2 to eliminate any effect of the copper. 200µl aliquots of both batches were incubated for 24 hours at 4, 10, 20, 30, 40, 50 and 62°C and degradation was assessed as described in Example 1 by size exclusion chromatography and measured as the extent of formation of "Peak C" based on the total eluted protein.

The results for % Peak C are given in Table 3 below:

TABLE 3

Temperature	% Peak C	
	Batch 1	Batch 2 + EDTA
4°C	0	0
10°C	0	0
20°C	0	0
30°C	0.47	0
40°C	2.71	0
50°C	60.1	0
62°C	72.36	1.12

Although no detectable Cu^{2+} was found in Batch 1, some degradation was apparent on incubation at 30 and 40°C with extensive degradation at 50 and 62°C. In the case of Batch 2 which contained detectable Cu^{2+}, minimal degradation was seen even at elevated temperature in the presence of EDTA. These results suggest the possibility that subdetectable levels of Cu^{2+} may accelerate the degradation of CAMPATH 1H.

EXAMPLE 4

The results of Example 1 were confirmed by a timed incubation at 62°C over a period of 24 hours using the same CAMPATH 1H antibody produced in CHO cells. The batch used was determined to contain 0.03µg Cu^{2+}/ml by atomic

absorption spectroscopy and 3ml of this batch containing 3.7mg/ml CAMPATH 1H in phosphate buffered saline was dialysed at +4°C for 24 hours against 3 x 2 litres 50mM ammonium hydrogen carbonate. 100 µl aliquots were incubated at 62°C with the following additions:

(i) 5µl 0.01M EDTA in water + 10µl 0.1M $CuCl_2.2H_2O$ in water;

(ii) 5µl 0.01M EDTA in water;

(iii) none.

The amount of EDTA added should have been sufficient to chelate any residual transition metal ions in the antibody but not sufficient to chelate the copper which is added in Sample (i).

50µl samples were withdrawn for analysis at the following times: 0, 1, 2, 3, 4, 5 and 24 hours. The samples were analysed as in Example 1 by size exclusion HPLC with the extent of formation of Peak C again being taken as a measure of the extent to which the antibody had been degraded. The results are shown in the following Table 4:

TABLE 4

Time hours	% Peak C		
	EDTA + Cu	EDTA	None
0	0	0	0
1	2.49	0	1.13
2	9.20	0	1.82
3	39.24	0	3.27
4	44.83	0	5.13
5	49.42	0	6.89
24	100	2.25	22.12

EXAMPLE 5

This example also used CAMPATH 1H produced in CHO cells of the type referred to in Example 1 (10.0mg/ml in phosphate buffered saline) and the batch having no detectable copper as measured by atomic absorption spectroscopy. A sample of this Campath 1H was dialysed at +4°C versus 50mM ammonium hydrogen carbonate and 100µl aliquots were incubated at 62°C for 24 hours with 10µl of increasing concentrations of $CuCl_2.2H_2O$ in water. The samples were analysed as in Example 1 by size exclusion HPLC with the extent of formation of "Peak C" again being taken as a measure of the extent to which the antibody had been degraded. The results are shown in the following Table 5:

TABLE 5

nMoles Cu per nMole CAMPATH 1H	% Peak C
0	1.61
0.018	8.09
0.037	11.41
0.074	13.61
0.145	17.59
0.293	22.84

The extent of degradation was found to increase with increasing molar ratio of Cu^{2+}/CAMPATH 1H. At ratios above 0.3 (data not shown), aggregation was seen with lower recovery of total protein.

EXAMPLE 6

This example also used CAMPATH 1H produced in CHO cells of the type referred to in Example 1 (1.0mg/ml in

phosphate buffered saline), the batch having been found to contain 0.19μg Cu^{2+}/ml as measured by atomic absorption spectroscopy. The sample thus had a high copper content (copper/CAMPATH 1H molar ratio 449 pMol Cu^{2+}/nMol CAMPATH 1H) and early stability studies showed that this batch was subject to substantial degradation on storage at 37°C.

The effect of incubation of this sample for up to four weeks at 37°C with and without the presence of 2mM EDTA is shown below in Table 6. The samples were analysed as in Example 1 by size exclusion HPLC with the extent of formation of "Peak C" again being taken as a measure of the extent to which the antibody had been degraded.

TABLE 6

Time (weeks)	% Peak C	
	2 mM EDTA	No EDTA
1	0.72	2.86
2	1.26	6.59
3	1.24	9.24
4	1.44	10.18
4 at +4°C	0.95	1.02

2 mM EDTA substantially decreases the decomposition of the CAMPATH 1H but does not totally inhibit it.

A sample of the same Campath 1H was dialysed at +4°C versus 50mM ammonium hydrogen carbonate and 100μl aliquots were incubated at 62°C for 24 hours with varying concentrations of EDTA. Again the samples were analysed as in Example 1 by size exclusion HPLC with the extent of formation of "Peak C" being taken as a measure of the extent to which the antibody had been degraded. The results of two separate experiments are shown in Tables 7 and 8 below:

TABLE 7

mM EDTA	% Peak C
0	6.86
0.1	1.03
1	1.38
2	1.12
3	1.26
4	1.04
10	1.20

TABLE 8

mM EDTA	% Peak C
0	7.47
0.0001	8.43
0.001	7.28
0.01	1.83
0.04	1.68
0.1	1.63

These results show that as little as 0.01mM EDTA effectively inhibits decomposition of CAMPATH 1H.

EXAMPLE 7

The effect of Cu^{2+} and of EDTA on the decomposition of various antibodies is shown in Table 9 below. All samples were incubated at 4°C and at 62°C for 24 hours in the absence of any additives and at 62°C for 24 hours in the presence of either Cu^{2+} (1mM $CuCl_2.2H_2O$ + 0.5mM EDTA) or EDTA

(1mM EDTA).

TABLE 9

Antibody	% Peak C			
	4°C No EDTA	62°C No EDTA	62°C + Cu^{2+}	62°C + EDTA
IgG1	0.54	1.58	5.59	1.1
C1H	0	2.49	27.98	0
CD4	0.4	1.91	21.52	1.84
IgG2	0	1.81	3.77	0

IgG1 = mouse monoclonal IgG_1 antibody, 1mg/ml in phosphate buffered saline;

C1H = CAMPATH 1H of the type described in Example 1, 1mg/ml in phosphate buffered saline;

CD4 = Humanised anti-CD4 monoclonal antibody having the same framework region as CAMPATH 1H and produced in CHO cells, 1mg/ml in phosphate buffered saline;

IgG2 = Mouse IgG_2 monoclonal antibody I-4139 commercially available from Sigma, supplied lyophilised from phosphate buffer and redissolved with water to 1mg/ml.

All samples show little or no decomposition at 4°C whereas there is some decomposition at 62°C which is increased by varying degrees by the presence of copper. Decomposition at 62°C is suppressed by EDTA.

EXAMPLE 8

A comparison between the effect of 2mM EDTA in phosphate buffered saline (pH 7.2) and 50 mM citrate (pH 6.0) on the stability of Campath-1H was carried out at various levels of copper. Campath-1H produced in CHO cells

of the type referred to in Example 1, the batch having no detectable copper as measured by atomic absorption spectroscopy, was diluted 1 in 10 by volume with phosphate buffered saline. 1 ml aliquots were dialysed against 1 litre of the following buffers:

(i) phosphate buffered saline, pH 7.2;
(ii) 2mM EDTA in phosphate buffered saline, pH 7.2;
(iii) 50 mM sodium citrate, pH 6.0.

Dialysis was carried out at 4°C with three changes over 16 hours. Protein concentration was then determined for the three samples by scanning between 340 and 200 nm using a buffer blank and taking the extinction coefficient A_{280} (0.1%) as 1.32. Protein concentrations of:

(i) 1.32 mg/ml
(ii) 1.20 mg/ml
(iii) 1.27 mg/ml

were determined.

200 µl aliquots of the antibody in the above buffers were then incubated with increasing concentrations of $CuCl_2.2H_2O$ (up to 20 mM) at 62°C for 24 hours (62°C being the optimal temperature for copper-induced cleavage of Campath-1H.) Samples (50 µl aliquots) were then analysed by size exclusion HPLC in the manner described in Example 1 and the various fractions integrated by cutting and weighing chromatograms of the A_{280}- absorbing peaks eluted from the column. In this case, results were recorded as % "peak B" (whole Campath-1H).

The results are set out in the following Table 10.

TABLE 10

Added Cu (mM)	% Peak B		
	PBS only	PBS+2mM EDTA	50mM Citrate
0	42.92	100	100
1	21.47	98.95	94.71
2.5	18.72	36.96	94.66
5.0	0	0	93.43
7.5	0	0	92.82
10	0	0	92.57
12.5	0	0	84.85
15	0	0	32.53
20	0	0	15.48

Cleavage of Campath-1H in phosphate buffered saline alone at pH 7.2 is relatively rapid on incubation for 24 hours at 62°C even in the absence of added copper. In phosphate buffered saline plus 2mM EDTA, pH7.2, cleavage is induced when greater than 1mM - copper is added. In 50mM - citrate, pH 6.0, cleavage takes place when copper in excess of 10mM is added.

EXAMPLE 9

A similar experiment to Example 8 also investigated the effece of varying the pH. Campath-1H produced in CHO cells of the type referred to in Example 1 in phosphate buffered saline, the batch having no detectable copper as measured by atomic absorption spectroscopy, was diluted 1:20 in phosphate buffered saline pH 7.2. Protein concentration was then determined as described in Example 8 and the samples diluted to a protein concentration of 2mg/ml with phosphate buffered saline pH 7.2 or phosphate buffered saline pH 6.0 and the pH was checked. Either 4µl 0.1M ~ trisodium citrate, pH 7.0 or 4µl 0.1 M-EDTA, pH 7.0 was added to 200µl aliquots of each of the Campath-1H samples (2mg/ml in phosphate buffered saline either pH 7.2 or pH 6.0) to give a final concentration of about 2mM with respect to citrate of EDTA.

Copper was added up to 3mM as 0 to 6µl aliquots of 0.1M $CuCl_2.2_2O$ per 200µl Campath-1H (2mg/ml) sample. 4µl water was added to samples without copper. Samples were incubated at 62°C for 24 hours, centrifuged to remove any precipitated material and 50µl aliquots analysed by size exclusion HPLC in the manner described in Example 8. Results, recorded as % "peak B" (whole Campath-1H) are set out in the following Table 11.

TABLE 11

Added Cu (mM)	% Peak B					
	PBS only		PBS+2mM EDTA		PBS+2Mm CIT	
	pH 7.2	pH 6.0	pH 7.2	pH 6.0	pH 7.2	pH 6.0
0	93.54	95.29	91.41	92.91	93.17	89.25
0.5	3.24	38.46	92.86	94.87	64.81	86.63
1.0	17.27	12.89	94.47	93.56	66.77	84.96
2.0	6.5	0	95.14	13	18.36	0.74
2.5	25	0	12.92	0	38.41	0.8
3.0	15.44	0	13.2	0	37.5	0.93

The above table shows the approximate stoichiometry of binding of Cu^{2+} by 2mM-EDTA and 2mM-citrate and the contributory effect of pH. 2mM-EDTA in phosphate buffered saline, pH 7.2, is the most effective in suppressing copper induced cleavage of Campath-1H. An approximate 1:1 stoichiometry of binding is indicated at pH 7.2. Copper concentrations in excess of 2mM cause cleavage of Campath-1H in 2mM EDTA.

CLAIMS:

1. A stabilised immunoglobulin composition comprising at least one immunoglobulin together with a stabilising amount of a chelator of copper ions.

2. A composition as claimed in Claim 1, wherein the immunoglobulin is of the class IgG.

3. A composition as claimed in Claim 1 or 2, wherein the immunoglobulin is a recombinant CDR-grafted antibody.

4. A composition as claimed in Claim 3, wherein the antibody is an antibody against the CD2, CD3, CD4, CD5, CD7, CD8, CD11a,b, CD18, CD19, CD25, CD33, CDw52 or CD54 antigen.

5. A composition as claimed in Claim 3, wherein the antibody is an antibody against the CDw52 antigen.

6. A composition as claimed in Claim 5, wherein the antibody is CAMPATH-1H.

7. A composition as claimed in any of Claims 1 to 6, wherein the chelator of copper ions is ethylenediamine tetraacetic acid.

8. A composition as claimed in any of Claims 1 to 6 wherein the chelator of copper ions is citrate ion.

9. A composition as claimed in any of Claims 1 to 8, in the form of a liquid preparation suitable for parenteral administration.

10. A composition as claimed in any of Claims 1 to 8, in

lyophilised form suitable for reconstitution into a liquid preparation suitable for parenteral administration.

11. Use of a chelator of copper ions to stabilise an immunoglobulin against degradation on storage.

12. Use according to Claim 11, wherein the chelator of copper ions is ethylenediamine tetraacetic acid.

13. Use according to Claim 11, wherein the chelator of copper ions is citrate ion.

14. Use according to any of Claims 11 to 13, wherein the antibody is a recombinant CDR-grafted antibody against the CDw52 antigen.

15. Use according to Claim 14, wherein the antibody is CAMPATH-1H.

16. A process for enhancing the stability of an immunoglobulin which comprises subjecting the immunoglobulin to a purification procedure capable of removing copper ions therefrom.

17. A process as claimed in Claim 16, wherein the purification procedure is dialysis versus potassium cyanide containing phosphate buffer followed by gel filtration to remove copper as copper cyanide.

18. A purified immunoglobulin substantially free from copper ions.

19. A purified immunoglobulin in which no copper can be detected by atomic absorption spectroscopy.

20. An immunoglobulin as claimed in Claim 18 or 19, which

is a recombinant CDR-grafted antibody against the CDw52 antigen.

21. An immunoglobulin as claimed in Claim 20, wherein the antibody is CAMPATH-1H.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/01970

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K39/395; G01N33/577; C12P21/08; //C07K3/28
G01N21/31

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	EP,A,0 391 526 (BIOPROBE INTERNATIONAL, INC.) 10 October 1990 see the whole document	1-21
A	BIOCHEMICAL PHARMACOLOGY vol. 21, no. 8, 15 April 1972, OXFORD, GB pages 1097 - 1105 M. CHVAPIL ET AL. 'Effects of selected chelating agents and metals on the stability of liver lysosomes.' see abstract see page 1099, line 12 - page 1101, line 10	1-21

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[10] Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 JANUARY 1993	08. 02. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	NOOIJ F.J.M.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	BIOTECHNOLOGY PROGRESS vol. 5, no. 3, September 1989, NEW YORK, USA pages 119 - 125 W. VELANDER ET AL. 'Process implications for metal-dependent immunoaffinity interactions.' see the whole document	1-21
P,X	US,A,5 087 695 (W. MCAULEY) 11 February 1992 see the whole document	1-21

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9201970 SA 65929

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 12/01/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0391526	10-10-90	US-A- 4933435	12-06-90
		CA-A- 2010835	05-10-90
		JP-A- 2290900	30-11-90
US-A-5087695	11-02-92	None	